Effect of Resistance to *Bacillus thuringiensis* Cotton on Pink Bollworm (Lepidoptera: Gelechiidae) Response to Sex Pheromone

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ABSTRACT Fitness costs associated with resistance to transgenic crops producing toxins from *Bacillus thuringiensis* (Bt) could reduce male response to pheromone traps. Such costs would cause underestimation of resistance frequency if monitoring was based on analysis of males caught in pheromone traps. To develop a DNA-based resistance monitoring program for pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), we compared the response to pheromone traps of males with and without cadherin alleles associated with resistance to Bt cotton (*Gossypium hirsutum* L.). When irradiated males from two hybrid laboratory strains with an intermediate frequency of resistance alleles were released in large field cages, the probability of capture in pheromone traps was not lower for males with resistance alleles than for males without resistance alleles. These results suggest that analysis of trapped males would not underestimate the frequency of resistance. As the time males spent in traps in the field increased from 3 to 15 d, the success of DNA amplification declined from 100 to 30%. Thus, the efficiency of a DNA-based resistance monitoring program would be improved by analyzing males remaining in traps for 3 d or less.

KEY WORDS *Bacillus thuringiensis* crops, cadherin, *Pectinophora gossypiella*, resistance detection, molecular monitoring

Transgenic cotton, *Gossypium hirsutum* L., that produces the *Bacillus thuringiensis* (Bt) toxin Cry1Ac has effectively controlled pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), in Arizona for nearly a decade (Carrière and Tabashnik 2001; Carrière et al. 2001a, 2003; Tabashnik et al. 2003, 2005b). Meanwhile, laboratory selection has produced several strains of pink bollworm resistant to Cry1Ac- and Cry1Ac-producing Bt cotton (Tabashnik et al. 2000, 2002, 2005a; Carrière et al. 2004a). In these laboratory-selected strains, resistance is associated with recessive mutations in a gene encoding a Cry1Ac-binding cadherin protein (Morin et al. 2003; Tabashnik et al. 2004, 2005a). Three resistance (*r*) alleles, each with a deletion in the cadherin coding sequence, have been identified (*r1*, *r2*, and *r3;* Morin et al. 2003). Morin et al. (2004) developed DNA-based methods for screening pink bollworm for such alleles. This molecular approach could improve resistance monitoring because it can rapidly detect single *r* alleles in heterozygotes, whereas conventional bioassays rely on detection of rare resistant homozygotes in laboratory-reared strains derived from field-collected individuals (Morin et al. 2004; Tabashnik et al. 2000, 2005b).

Monitoring insecticide resistance by testing males caught in pheromone traps has been a common practice (Haynes et al. 1987, Brewer and Trumble 1989, Cossentine and Jensen 1991, Shearer and Riedl 1994). Thus, traps baited with sex pheromone provide a convenient way to collect pink bollworm males for monitoring resistance. However, to estimate resistance frequency from trapped individuals, it is useful to assess whether the probability of capture is equal for resistant and susceptible individuals. Attraction to pheromone traps was higher for susceptible males than pyrethroid-resistant males of *Heliothis virescens* (F.) (Campanhola et al. 1991) and azinphosmethyl-resistant males of *Phyllonocrycter elamaella* (Doganlar & Mutuuara) (Shearer and Riedl 1994). In the green peach aphid, *Myzus persicae* (Sulzer), reduced responses to alarm pheromones were associated with target site resistance (*kdr*) to pyrethroids and DDT as well as resistance to organophosphates caused by amplified carboxylesterase (Foster et al. 1999, 2003). In *Anopheles gambiae* (Giles) and *Anopheles stephensi* (Liston), resistance to γ-hexachlorocyclohexane and dieldrin affected activity patterns and mating responses (Rowland 1991a,b). If fitness costs or other factors reduce the capture probability of resistant in-

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dividuals relative to susceptible individuals, analysis of trapped individuals could underestimate resistance frequency.

In pink bollworm, resistance to Bt cotton is associated with fitness costs affecting several life history traits, including survival on non-Bt cotton plants, overwintering survival, and paternity (Carrière et al. 2001b,c, 2004a,b, 2005; Higginson et al. 2005). Thus, we hypothesized that fitness costs might reduce responses to pheromone traps by resistant or heterozygous males relative to susceptible males. In a previously reported experiment with large cages in the field, the probability of capture in pheromone traps for irradiated male pink bollworm was 56% for a susceptible strain (APHIS) and 37% for a strain resistant to Bt cotton (AZP-R) (Staten et al. 2002). Because these two strains differed in origin and other traits besides resistance, the reduced capture rate for resistant males might have been caused by factors other than resistance. Furthermore, the comparison of Staten et al. (2002) did not address the response of heterozygotes to pheromone traps. It is crucial to know the responses of heterozygotes, because most resistance alleles occur in heterozygotes when resistance is rare (Tabashnik 1997, Sisterson et al. 2004).

In this study, we compared the response to pheromone traps among males with different cadherin genotypes within two hybrid strains of pink bollworm. Because males with zero, one, or two *r* alleles occurred in both strains, the effects of differences unrelated to cadherin genotype were greatly reduced or eliminated. To determine how often traps should be collected for resistance monitoring, we also evaluated DNA quality in males left in traps in the field for 1–15 d.

Materials and Methods

Pink Bollworm Strains. Larvae were reared on wheat germ diet. Moths were from two hybrid strains, MOV97-H1 and SAF97-H1. The MOV97-H1 hybrid strain was created by pooling offspring from reciprocal crosses between the laboratory-selected resistant strain MOV97-R (F57 and F58) and its unselected parent strain (F62 of MOV97): 300 MOV97-R males × 300 MOV97 females and 300 MOV97-R females × 300 MOV97 males. The SAF97-H1 hybrid strain was created with parallel reciprocal crosses between the laboratory-selected resistant strain SAF97-R (F55) and its unselected parent strain (F60 and F61 of SAF97). Tabashnik et al. (2005a) describes the origin, maintenance, and survival on Bt and non-Bt cotton of MOV97, MOV97-R, SAF97, and SAF97-R.

Cadherin resistance alleles *r1* and *r3* occur in MOV97-R; *r1* and *r2* occur in SAF97-R (Morin et al. 2003; Tabashnik et al. 2004, 2005a). When the hybrid strains were created, the frequency of *r* alleles was 1.0 in MOV97-R and SAF97-R. The SAF97 and MOV97 strains contained some *r* alleles because the frequency of resistance was high in the field populations from which they originated (Tabashnik et al. 2000, Carrière et al. 2004a). The frequency of *r* alleles in the F1 progeny was 0.52 for SAF97-H1 and MOV97-H1 (J. Williams, unpublished data). Before experimental releases, moths interbred for four generations in each hybrid strain, which reduced linkage disequilibrium between resistance alleles and other alleles potentially affecting the response to pheromone traps (Falconer 1981).

Marking, Irradiation, and Prerelease Sampling of Males. Males from the fifth generation of the hybrid strains were released in field cages in two replicates (see below). For each replicate, newly emerged unmated males were placed in five containers (80 males per container for SAF97-H1 and 100 males per container for MOV97-H1) with access to a 10% honey solution. When males were put in containers, an independent random sample of 250 males from each strain was collected and stored in ethanol at −80°C for subsequent genotyping (see below). We genotyped 400 prerelease males (100 of each hybrid strain from each of two replicates).

Moth containers were taken in coolers from the University of Arizona (Tucson, AZ) to the USDA–Animal and Plant Health Inspection Service (APHIS) Laboratory (Phoenix, AZ) where they were marked with powdered fluorescent dye and irradiated. Males from MOV97-H1 were marked green and those from SAF97-H1 were marked pink to allow sorting of recaptured individuals. The irradiation rate was 10 kRad for 2 min, which is sufficient to avoid any fertile F2 progeny (Staten et al. 2002). MOV97-H1 and SAF97-H1 males <4 d old were irradiated in the morning and released in field cages from 1700 to 1800 hours on the same day.

Release in Field Cages and Capture. Five field cages (4 m in width by 8 m in length by 2 m in height) were placed over three rows of cotton (≈1 m in height) at the University of Arizona Campbell Agricultural Center (Tucson, AZ). We used five cages in the first replicate and four in the second replicate, because a thunderstorm damaged one cage between the first and second replicates. Releases for the first and second replicate were respectively done on 8 and 29 August 2003. No other cotton was grown within >10 km of this site. Although moths were irradiated, we also destroyed the cotton in cages immediately after the second replicate to reduce the chances of escape of resistant insects.

Two pheromone traps (Pherocon IIID delta traps (Trécé Inc., Salinas, CA), each baited with a single rubber septa impregnated with gossyplure (Trécé Pink Bollworm Lure, Trécé Inc.; 4 μg of gossyplure per septa), were placed ≈2 m from the north and south side of each cage. Males from MOV97-H1 and SAF97-H1 were released in the center of each cage by gently emptying the content of each container (one male per strain) on a separate cotton plant. After each release, pheromone traps were collected and brought to the laboratory for inspection every 3 d for 15 d. New traps with fresh pheromone lures were put in the cages immediately after each collection. Recaptured moths were sorted by strain based on the color of fluorescent

powder and stored in ethanol at −80°C for subsequent genotyping (see below).

Temporal Change in DNA Quality. On 4 June 2003, 200 live males from SAF97-R and MOV97-R were put in delta traps (25 males per trap and four traps per strain) ≈1 m above ground in an open field at the University of Arizona Campbell Agricultural Center. Traps with males were placed in the field in the morning. Sixteen males per strain were randomly collected (four per trap) in the morning 1, 2, 3, 5, 10, and 15 d later. The Arizona Meteorological Network (Brown and Russell 1995) was used to record daily minimum and maximum temperature and average relative humidity during this experiment.

To simulate a field collection, males taken from traps were placed in microcentrifuge tubes, and the tubes were kept in a cooler for 3 to 4 h before being stored in ethanol at −80°C. For each collection date, between six and 11 males were randomly subsampled from the strains and analyzed to assess whether DNA could be amplified successfully with polymerase chain reaction (PCR).

DNA Analyses to Determine Cadherin Genotype. DNA was extracted using the previously described DNAzol (Molecular Research Center, Cincinnati, OH) method (Tabashnik et al. 2005a). Individuals were genotyped using previously described PCR reactions (Morin et al. 2004). Prerelease males from MOV97-H1 were tested for *r1* and *r3;* those from SAF97-H1 were tested for *r1* and *r2.* Males captured in cages were analyzed for *r1*, *r2*, and *r3.* For the DNA viability experiment, a conserved region of the cadherin gene was amplified using PCR primers 3324, 3963, and 4074 (Higginson et al. 2005).

Statistical Analyses. To investigate whether costs affect the capture of males in pheromone traps, we compared three variables between prerelease and captured males: the frequency of *r* alleles; the frequency of *rr*, *rs*, and *ss* genotypes; and the frequency of individual genotypes (e.g., *ss*, *r1r1*, *r1r3*, *r3r3*, *r1s*, and *r3s* in MOV97-H1).

Change in Frequency of Resistance Alleles. To test the hypothesis that resistance alleles are costly, the change in *r* allele frequency between prerelease and captured males was calculated for the two replicates of each strain. Pooling the changes in frequency estimated for the strains, a one-sample *t*-test was used to assess whether the average change in *r* allele frequency differed from zero.

Change in Frequency of the *rr*, *rs*, and *ss* Genotypes. To test the hypothesis that capture of genotypes with resistance alleles is lower than in genotypes without resistance alleles, individuals with two, one, or no resistance alleles were categorized as *rr*, *rs*, and *ss* genotypes, respectively. Pearson chi-square tests were used to assess changes in frequency of the genotypes between prerelease and captured males. Such tests were performed using data from each strain and replicate (four tests performed), or data were pooled for the two replicates conducted for each strain (two tests performed).

To further assess differences in capture of the genotypes, we compared the estimated capture proportion of the genotypes in each strain. To estimate the capture proportion of each genotype, we first calculated the number of individuals from each genotype released in field cages, by multiplying the total number of individuals released by the frequency of each genotype in prerelease moths. Second, we estimated the number of captured individuals from each genotype, by multiplying the total number of males captured by the frequency of the genotypes estimated in captured individuals. The estimated capture proportion of each genotype was obtained by dividing the number of captured individuals for each genotype by the number of released individuals for each genotype. A two-way analysis of variance (ANOVA) was used to assess whether the mean capture proportion differed between replicates and among genotypes in each strain. Contrasts between means were used to test the a priori hypothesis that the capture proportion of the *rr* or *rs* genotype was lower than the capture proportion of the *ss* genotype.

Change in Frequency of Individual Genotypes. To more specifically assess capture differences among individual genotypes, Pearson chi-square tests were used to assess changes in frequency of the genotypes between prerelease and captured males, either by using data from each strain and replicate (four tests performed) or by pooling data for the two replicates conducted for each strain (two tests performed). To further assess differences in capture proportions of individual genotypes, we used the method outlined above to compare the estimated capture proportion of each genotype in a strain. A two-way ANOVA was used to assess whether the mean capture proportion differed between replicates and among genotypes. Contrasts between means were used to compare the capture proportion of each genotype with one or two *r* alleles to the recapture proportion of *ss.*

Results

Release and Capture of Males. For MOV97-H1 replicates 1 and 2, respectively, 500 and 400 moths were released; 163 and 270 males were captured. For SAF97-H1 replicates 1 and 2, respectively, 400 and 320 moths were released; 140 and 170 moths were captured. Most males were captured 1–3 d after release. Of the 433 MOV97-H1 males captured, an average of 81% were caught 1–3 d after release, 13% were caught 4–6 d after release, and the remaining 6% were caught 7–15 d after release. Of the 310 SAF97-H1 males captured, an average of 69, 18, and 13% were caught 1–3, 4–6, and 7–15 d after release, respectively. For MOV97-H1, only males caught 1–3 d after release were genotyped. For SAF97-H1, an average of 80, 17, and 3% of the captured moths genotyped were from 1–3, 4–6, and 7–9 d after release, respectively.

Change in Frequency of Resistance Alleles. The average change in frequency of the resistance alleles between prerelease and captured males was not significantly different from zero (average, 0; 95% CI,

Table 1. Number of moths genotyped (*N*) for each replicate of prerelease and captured moths from the strains MOV97-H1 and SAF97-H1

Strain	Rep	Sample	*N*	*r* Allele frequency	Genotype			*P*
					rr	*rs*	*ss*	
MOV97-H1	1	PRE	100	0.48	28 (23)	41 (50)	31 (27)	0.64
		CAP	112	0.50	29 (28)	53 (56)	30 (28)	
	2	PRE	100	0.49	22 (24)	54 (50)	24 (26)	0.70
		CAP	112	0.51	24 (29)	66 (56)	22 (27)	
SAF97-H1	1	PRE	100	0.41	15 (17)	52 (48)	33 (35)	0.42
		CAP	107	0.40	20 (17)	46 (51)	41 (39)	
	2	PRE	100	0.48	24 (23)	49 (50)	27 (27)	0.73
		CAP	107	0.45	21 (22)	54 (53)	32 (32)	

CAP, captured; PRE, prerelease; Rep, replicate.

Also shown is the overall frequency of the resistance alleles, the observed and expected number of each genotype (expected numbers based on Hardy–Weinberg equilibrium in parentheses), and the probability value (*P*) assessing differences in frequency of the genotypes between the PRE and CAP moths in each replicate.

−0.039–0.039) ($t = 0$, df = 3, $P = 1$). Thus, *r* and *s* alleles had similar effects on the probability of capture in pheromone traps.

Change in Frequency of the *rr*, *rs*, and *ss* Genotypes. Frequency of the genotypes did not differ significantly between prerelease and captured moths (Table 1). After pooling data for the two replicates of each strain, there was still no difference in the frequency of the genotypes between the prerelease and captured moths (MOV97-H1: $\chi^2 = 1.51$, $P = 0.47$; SAF97-H1: $\chi^2 = 0.85$, $P = 0.65$).

In MOV97-H1, the mean capture proportion of the genotypes was significantly lower in the first than in the second replicate (0.32 versus 0.65: $F = 128.25$; df = 1, 2; $P = 0.0077$). No significant difference among the capture proportions of the genotypes was evident ($F = 7.70$; df = 2, 2; $P = 0.11$). However, contrasts between means indicated a greater capture proportion in the *rs* than *ss* genotype (Fig. 1) ($P = 0.059$), although the capture proportion of the *rr* and *ss* genotype was similar ($P = 0.22$).

The rank order of the capture proportion of the genotypes was similar in the two replicates involving MOV97-H1 (Spearman Rho correlation = 1, $P < 0.0001$, $N = 3$), further indicating that differences among the capture proportions of the genotypes were repeatable. Nevertheless, it seems possible that random sampling was influential in generating the significant difference between the *rs* and *ss* genotypes (Fig. 1). Specifically, inspection of deviations from Hardy–Weinberg expectations in *rs* individuals of the first replicate (Table 1) indicates that the frequency of this genotype was substantially lower than expected in prerelease individuals, and still lower than expected in captured individuals. Accordingly, random sampling could have led to overestimating the capture proportion of this genotype in the first replicate. Although this trend was not present in the second replicate (Table 1), we conclude that the significant difference between the capture proportion of the *rs* and *ss* genotype (Fig. 1) may have been caused at least in part by random sampling effects.

In SAF97-H1, the mean capture proportion of the genotypes did not differ significantly between the first and second replicate (0.37 versus 0.52) ($F = 3.49$; df = 1, 2; $P = 0.20$). Capture proportions did not differ among genotypes ($F = 0.43$; df = 2, 2; $P = 0.70$; all contrasts between means, $P \geq 0.48$) (Fig. 1) nor was there any significant correlation between the rank order of the capture proportion of the genotypes in the two replicates (Spearman Rho = −0.50, $P = 0.67$, $N = 3$).

In summary, there was no evidence for the presence of costs associated with resistance to Bt cotton in the *rr* and *rs* genotypes. Although repeatable differences in the probability of capture of the genotypes were observed across replicates in MOV97-H1, random sampling may have been partially responsible for the significant difference between the capture proportion of the *rs* and *ss* genotype.

Change in Frequency of Individual Genotypes. The frequency of the genotypes did not differ significantly between the prerelease and captured males (Table 2) (*P* values ≥0.21 for the four comparisons). When data were pooled for each strain, there was no difference in the frequency of the genotypes between the prerelease and captured moths (MOV97-H1: $\chi^2 = 7.42$, $P = 0.19$; SAF97-H1: $\chi^2 = 4.00$, $P = 0.55$).

In MOV97-H1, the mean capture proportion of the genotypes was significantly lower in the first than in the second replicate (0.32 versus 0.66) ($F = 23.61$; df = 1, 4; $P = 0.0083$). There was a marginally significant difference among the capture proportions of individual genotypes ($F = 4.40$; df = 5, 4; $P = 0.088$). Contrasts between means indicated a higher capture proportion in *r1r3* ($P = 0.057$) and *r1s* ($P = 0.060$) (Fig. 2) than in *ss.* No significant differences between the capture proportion of *r1r1*, *r3r3*, *r3s*, and *ss* were evident ($P \geq 0.42$).

The rank order of the capture proportion of the genotypes was similar in the two replicates involving MOV97-H1 (Spearman Rho correlation = 0.83, $P = 0.037$ $N = 5$) (*r1r1* could not be included in this analysis because *r1r1* individuals were not detected in prerelease moths in the second replicate; see Table 2), again indicating repeatable differences among the capture proportions of the genotypes. However, there was also a trend for a lower than expected frequency of the *r1s* genotype (based on Hardy–Weinberg deviations) in prerelease individuals of the first replicate

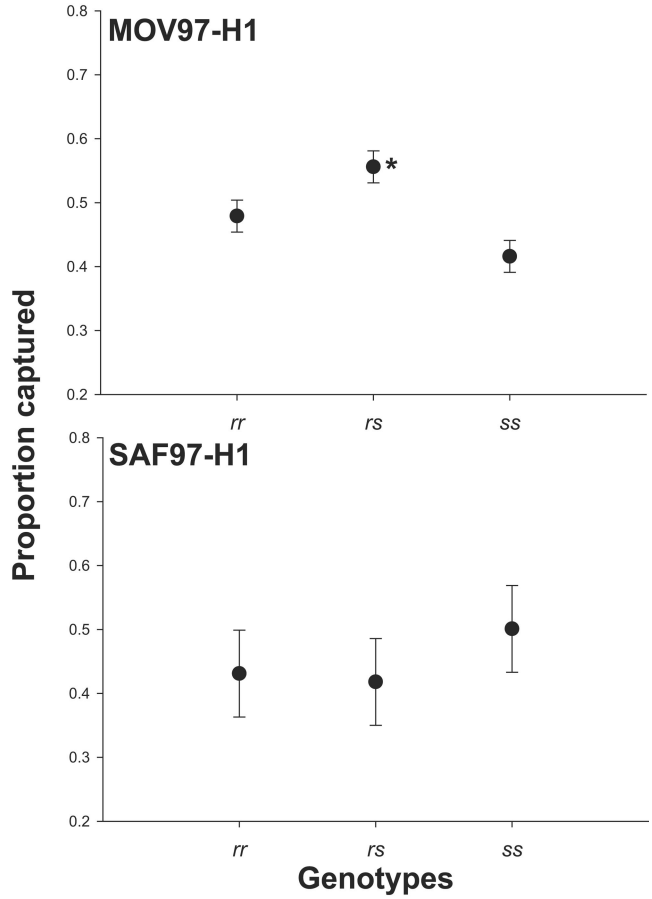

Fig. 1. Proportion capture ± SE in pheromone traps of the *rr*, *rs*, and *ss* genotypes of MOV97-H1 and SAF97-H1. Proportion with a star was significantly different from proportion capture of the *ss* genotype ($P = 0.06$).

(Table 2). Thus, as for the difference between *rs* and *ss* (Fig. 1), the difference between the response of the *r1s* and *ss* genotypes (Fig. 2) may have been overestimated. In contrast, the observed genotypic frequencies of *r1r3* was never lower than expected in prerelease individual (Table 2), suggesting that the capture proportion of this genotype was actually greater than in the *ss* genotype.

In SAF97-H1, the mean capture proportion of the genotypes did not differ significantly between the first and second replicate (0.42 versus 0.57) ($F = 0.906$; df = 1, 4; $P = 0.39$). Capture proportions did not differ among genotypes ($F = 0.16$; df = 5, 4; $P = 0.96$; all contrasts between means, $P \geq 0.62$) (Fig. 2) nor was there any significant correlation between the rank order of the capture proportion of the geno-

Table 2. Observed and expected number of each genotype (expected numbers based on Hardy–Weinberg equilibrium in parentheses) within each replicate of prerelease and captured moths

Strain	Rep	Sample	Genotype					
			r1r1	*r1r3*	*r3r3*	*r1s*	*r3s*	*ss*
MOV97-H1	1	PRE	5 (3)	12 (11)	11 (10)	12 (18)	29 (32)	31 (27)
		CAP	2 (4)	17 (13)	10 (10)	22 (22)	31 (34)	30 (29)
	2	PRE	0 (2)	9 (9)	13 (13)	17 (13)	37 (37)	24 (26)
		CAP	0 (4)	15 (13)	9 (12)	25 (20)	41 (36)	22 (27)
			r1r1	*r1r2*	*r2r2*	*r1s*	*r2s*	*Ss*
SAF97-H1	1	PRE	0 (1)	5 (5)	10 (11)	10 (9)	42 (40)	33 (36)
		CAP	2 (2)	10 (8)	8 (8)	15 (17)	31 (34)	41 (38)
	2	PRE	4 (6)	16 (12)	4 (6)	25 (25)	24 (25)	27 (27)
		CAP	5 (6)	9 (11)	7 (5)	30 (27)	24 (26)	32 (32)

See Table 1 for designation of strains and number of moths genotyped for each replicate.

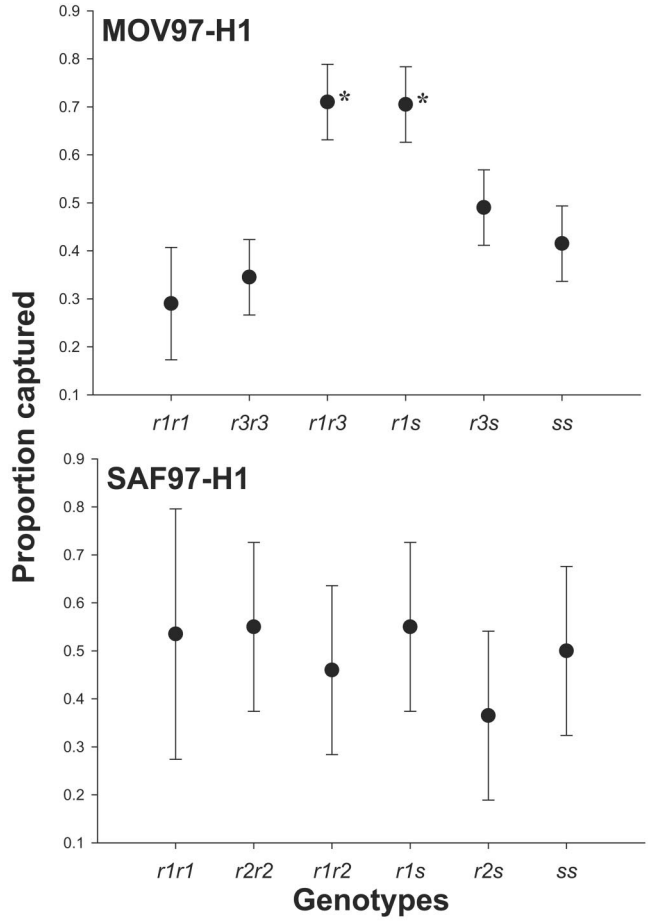

Fig. 2. Proportion capture ± SE in pheromone traps of the cadherin genotypes of MOV97-H1 and SAF97-H1. Proportions with a star were significantly different from proportion capture of the ss genotype ($P \leq 0.06$).

types in the two replicates (Spearman Rho = −0.30, $P = 0.62$, $N = 5$) (*r1r1* could not be included in this analysis because *r1r1* individuals were not detected in prerelease moths from the second replicate; see Table 2).

In summary, there was no evidence for the presence of costs affecting the capture proportion of individual genotypes. However, individuals from *r1r3* seemed to have a greater probability of capture in pheromone traps than ss individuals in MOV97-H1.

Temporal Change in DNA Quality. During this experiment, the average minimum and maximum temperature (Centigrade) and the average relative humidity (percentage) was 17.0 (95% CI, 15.5–18.5), 37.6 (36.5–38.8) and 18.0 (16.5–19.4), respectively. Approximately 75% of the moths placed in traps in the morning died by the next morning. None were alive after being in traps for 2 d. The rate of successful PCR amplification of DNA extracted from moths was 100% for moths that had spent less than 3 d in traps and decreased to 30% at 15 d (Table 3).

Discussion

The goal of this study was to evaluate whether resistance to Bt cotton affects the response of pink bollworm males to sex pheromone. We found that within two hybrid strains of pink bollworm, the probability of being caught in pheromone traps was not higher for susceptible males than for males with one or two cadherin alleles linked with resistance to Bt cotton. Thus, although the field cages (4 m in width by 8 m in length by 2 m in height) may not have been large enough to allow expression of the full range of

Table 3. Percentage of DNA samples amplified with PCR in moths having spent between 1 and 15 d in traps in the field

Time in traps (d)	No. moths assayed	Amplification (%)
1	8	100
2	8	100
3	6	100
5	10	80
7	11	82
10	8	75
15	10	30

behaviors involved in pheromone response, our results suggest that molecular analyses of pink bollworm males obtained from pheromone traps will not underestimate the frequency of cadherin-based resistance to Bt cotton. In our experiments, the probability of capture in pheromone traps could have been affected by male survival and attraction to traps. Although we cannot separate effects of these two factors, the results show that their combined effect did not differ as a function of male cadherin genotype within strains.

Our results contrast with a previous study in which the probability of capture in pheromone traps was 1.5-fold higher in a homozygous susceptible strain (APHIS) than in an unrelated, homozygous resistant strain (AZP-R) (Staten et al. 2002). The dissimilarity between studies was likely caused by differences between strains other than resistance, which were greatly reduced or excluded in the current study but not in the comparison between unrelated strains.

We used two types of statistical analyses to compare pink bollworm response to pheromone traps. The first analysis was more general than the second analysis, because it compared the frequency of all genotypes before and after release: it did not reveal any significant among-genotype differences in male response to pheromone traps. The second analysis compared the estimated capture proportion of genotypes with resistance alleles to the capture proportion of the ss genotype. After taking into account possible sampling effects on the outcome of the comparisons between pairs of genotypes (through consideration of deviations from Hardy–Weinberg expectations), we unexpectedly found that individuals from the genotype *r1r3* seemed to have a greater probability of capture in pheromone traps than individuals from the *ss* genotype in MOV97-H1. Because the two methods assessed different questions (general versus specific planned comparisons), they were not expected to yield identical findings (Ramsey and Schafer 2002).

Linkage disequilibrium between cadherin mutations and alleles on other chromosomes is expected to be minimal after four generations of random mating (Falconer 1981). However, linkage with alleles on the same chromosome as the cadherin locus can persist for a longer time (Falconer 1981). Nevertheless, linkage between the cadherin mutations and alleles potentially affecting the response to pheromone traps is expected to be the same in the *r1r1*, *r3r3*, and *r1r3* genotype. Thus, that the response of these genotypes was significantly different in MOV97-H1 (*r1r3* versus *r1r1* and *r3r3* contrast, $P = 0.020$) (Fig. 2) provides evidence for a direct effect of the cadherin mutations on the male response to pheromone traps.

Resistance to insecticides is generally expected to induce fitness costs (Carrière et al. 1994, McKenzie 1996, Raymond et al. 2001). However, resistance has been occasionally associated with increased fitness in absence of insecticides (Arnaud and Haubruge 2002, McCart et al. 2005). Here, the positive effect of the cadherin mutations on the capture proportion of the r1r3 genotype was only apparent in one strain (Fig. 1), indicating that the phenotypic effect of the cadherin mutations depended on genetic background (McKenzie et al. 1982, McKenzie 1996, Remold and Lenski 2004). Because it is unclear at this point whether the among-genotype variation in the response to pheromone traps in MOV97-H1 would result in fitness differences in the field, it is premature to conclude that the cadherin mutations had positive effects on a pink bollworm fitness component.

In conjunction with bioassays (Tabashnik et al. 2005b), DNA-based screening is underway to monitor for pink bollworm resistance to Bt cotton in Arizona. Samples have been obtained in many populations for several years by collecting larvae in bolls and males in pheromone traps. The present findings indicate that molecular analysis of the latter samples will not underestimate the frequency of cadherin-based resistance and that pheromone traps should be monitored every 3 d to maximize success.

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